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Antimicrobial Potential of *Passiflora alata* and *Piper methysticum* hydroalcoholic extracts, Phytotherapies of Anxiolytic-like Activity

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Natural products are important source for new drug development antimicrobial therapy research. The variety of active molecules and the lack of resistance-inducing mechanisms of action are the main attractive features of these compounds that inspire the scientific community to explore them as potential alternatives to overcome resistance to traditional synthetic drugs.

In an attempt to explore potential antimicrobial phytotherapeutic preparations that are not strongly linked with this biological feature, here we show an investigation using plant extracts commonly employed in the phytotherapeutic management of anxiety disorders and sleep disturbances. The occurrence of these psychiatric disorders has been largely reported in the latest years, and they can affect any age group independently of race and economic status¹.

Here we explored *Passiflora alata* and *Piper methysticum* extracts, which are widely used in Brazil as alternative therapies for sleep disturbances. *P. alata* (Passion fruit) is an important crop largely explored commercially for pulp juice and tea production. The plant is generally grown in tropical and subtropical regions of planet². The pulp juice is appreciated in several countries and varied commercial phytotherapeutic formulations can be purchased without medical prescription^{2,3}.

Piper methysticum (Kava) is a psychotropic plant from South Pacific with recognized anxiolytic-like activity. The pharmacological properties of kava are postulated to include blockade of voltage-gated sodium ion channels, enhanced ligand binding to gamma-aminobutyric acid type A (GABA-A) receptors, and reduced neuronal reuptake of

noradrenaline and suppression of the synthesis of the GABA-A antagonist thromboxane A₂^{1,4}.

The antimicrobial activity of these extracts is poorly documented, and considering the large acceptance of these by the population to support the treatment of anxiety disorders and sleep disturbances, we hypothesized if they would be effective against *Escherichia coli* and *Staphylococcus aureus* strains. *P. alata* hydroalcoholic leaf extract (PHLF - Pharmanostra, Brazil) and *P. methysticum* hydroalcoholic rhizome extract (PHRE - Fagron, Brazil) were filtrated, rotavaporized at 50 °C to form a brown grease, and stored in amber bottles at 4 °C until used. Stock solutions of 4 mg/mL were prepared in DMSO (Synth) and used immediately.

Clinical isolates of *S. aureus* (10 strains, from catheter tips) and *E. coli* (10 uropathogenic strains), from the microorganisms collection of the Santo Agostinho Institute were used. Each isolate was prepared in a saline suspension and tested with the VITEK 2 system (version R04.02, bioMérieux) using Gram-positive and Gram-negative cards, according to the manufacturer's instructions. Prior to the commencement of the study, all strains were activated overnight in BHI broth (Difco) at 37 °C.

To investigate the minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) of PHLF and PHRE, we performed broth microdilutions assays in non-treated 96-wells polystyrene plates with Mueller Hinton broth, as previously described⁵, with slight modifications. Organisms were used in 0.5 MacFarland turbidity scale and were exposed to the extracts in serial dilutions ranging from 2 mg/mL to 15.6 µg/mL. After, plates were incubated overnight at 37 °C. MIC was determined using resazurine staining (0.1 g/L), and MBC was determined by dropping aliquots of 10 µL onto the surface of Mueller Hinton agar (Difco) plates. After overnight incubation, the formation of colonies was observed. The MBC for each extract was the lowest concentration that fully inhibited bacterial growth in agar plates.

Results are summarized in table 1. PHLF was equally potent for the tested strains. PHRE, on the other hand, was poorly effective against *S. aureus* strains. Unfortunately, because of the high value of MIC for PHRE for *S. aureus* strains, it was not possible to determine the MBC of this extract. PHLF MIC value for *E. coli* strains was eight times lower than PHRE, and 64 times lower than PHRE for *S. aureus* strains.

Table 1 – MIC and MBC results for *S. aureus* and *E. coli* strains.

Plant Extract	MIC value (µg/mL) <i>S. aureus</i>	MBC value (µg/mL) <i>S. aureus</i>
PHLF	15.6	125
PHRE	1	ND
Plant Extract	MIC value (µg/mL) <i>E. coli</i>	MBC value (µg/mL) <i>E. coli</i>
PHLF	15.6	250
PHRE	125	1

PHLF: *P. alata* hydroalcoholic leaf extract. PHRE: *P. methysticum* hydroalcoholic rhizome extract. ND: not determined.

As mentioned, the antimicrobial activity of these plant extracts is poorly known. Kavalactones such as kavain, dihydrokavain, methysticin, and dihydromethysticin are among the main phytochemicals of PHRE^{1,4}, and preparations of the rhizome were reported to inhibit pathogenic plant fungus growth⁴. The antimicrobial activity of *Passiflora* species is poorly described, and in this study, PHLE was effective against *E. coli* and *S. aureus* strains.

In conclusion, *P. alata* extract was more effective than *P. methysticum* extract against *E. coli* and *S. aureus*. These extracts, although used mostly for anxiety disorders, have interesting antimicrobial properties. In further studies, our group shall explore other antimicrobial parameters of these extracts.

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